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Analysis of melamine contamination in meat and dairy products commercialized in the western part of Romania

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Abstract

Due to some highly publicized incidents a few years ago, melamine is considered a food contaminant which may occur accidentally or intentionally in certain types of food, and long-term consumption of melamine can severely affect the health of consumers.

This study analyzed 6 samples of chicken meat and 7 powder milk samples from commercially available food products, with origin in different geographic regions of Romania and packed in different types of packaging. The purpose was to determine melamine concentration and to observe the influence of origin or type of packaging on the melamine content in the analyzed samples. Such studies are part of the current efforts for improving food analysis and control strategies in alignment with EU policies in terms not only of legislation but also of verification and control actions. The screening of chicken meat and powder milk samples showed the presence of melamine in all samples with values lower than the maximum allowed limit for food of 2.5 ppm, indicating that the level of melamine is not the result of intentional addition of melamine in these food products. Analysis of the various factors influence on the melamine concentration in the analyzed samples showed that melamine content depended on geographical region and type of packaging but larger studies are needed for establishing statistical significant correlations between the geographical region, type of packaging and the shelf life period.

Keywords: melamine, contamination, chicken meat, powder milk, nitrogen content

1. Introduction

Food contamination, accidental or deliberate, is one of the major food safety issues. In order to avoid contamination, food production is closely monitored throughout the production chain and the laws and policies developed for this purpose contribute to create a framework for the development of both global and european food trade, while protecting public health and wherever possible the animals, plants and the environment. Due to some food safety incidents in recent years which were highly mediatized, melamine is considered a substance that should not occur in food either accidentally or intentionally [1].

Melamine (1,3,5-Triazine-2,4,6-triamine) is an organic substance with molecular formula $C_3H_6N_6$ (Figure 1).

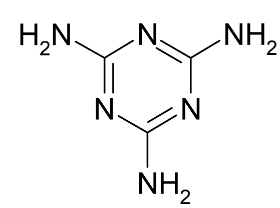

Figure 1. Melamine structure

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Melamine is a nitrogen base with a nitrogen content of 66%, which makes it possible to use melamine for falsification of food protein content, food proteins being determined by a non-specific method (Kjeldahl method) which determine the total nitrogen content from a food sample (both protein and non-protein nitrogen). Thus, the melamine added to the sample increases the nitrogen content percentage in the analyzed sample while the protein content remains unchanged.

In 2007 melamine came to public attention in North America, following complaints about cats and dogs food, followed by the withdrawal of large quantities of these types of food of the market.

In 2008, in China were detected cases of melamine contamination of fresh milk, some powder milk formulas, eggs and other products [3-9]. In these cases, melamine and melamine related products have been intentionally added to protein ingredients or foods intended for human or animal consumption.

Melamine and its deaminated derivative, the cyanuric acid have been added to food in order to falsely increase their protein content by increasing the percentage of nitrogen content in the analyzed food.

People are exposed to melamine from different sources, such as:

- pesticide decomposition (decomposition of cyromazine from pesticides)
- food contamination by migration of melamine and its analogues from the food plastic packaging
- intentionally adding of melamine (illegal) in different food products (eg milk) in order to increase the amount of nitrogen in the product
- consumption of animal products (milk, eggs, meat) from animals that received melamine in their food.

Studies of melamine migration from plastic packaging using different migration conditions (temperature, different solvents used in foods industry) showed that the melamine levels varies very widely depending on the used conditions [10,11,12]. Also, several studies showed that the levels of foods contamination with melamine consecutively to spontaneous migration from plastic packaging are lower than 1 mg/kg due to experimental conditions (high temperature) [13].

Melamine from pesticides (from cyromazine), which may contaminate the plants (potatoes, beans, tomatoes, lettuce) during plant development, had values lower than 1 mg/kg [14,15].

Toxicity

Trichloromelamine which is used to clean kitchen equipments and utensils (except for milk processing equipments) may decompose to melamine, which can reach food products. In these circumstances, values of 0.14 mg melamine/kg were reported, considering that all kitchen tools contained trichloromelamine [13].

The European Union authorizes a melamine maximum permissible dose of 0.5 mg/kg/day, Canada allows 0.35 mg/kg/day and in the United States, the FDA (U.S. Food and Drug Administration) originally set a limit of 0.63 mg/kg/day and then reduced this limit to 0.063 mg/kg/day. These limit values are calculated using short-term laboratory studies on animals and it's obvious that the results translation from animal studies to human risk assessment entails a degree of uncertainty which is high enough according to some experts [16,17].

In 2008, the World Health Organization (WHO) estimated that the daily intake of melamine to which a person can be exposed without risk is 0.2 mg/kg/day for all people including children. The safe level of melamine in solid food was calculated by the FDA assuming that an average sized person (60 kg) has a dietary intake of 3 kg food/day, from which 1.5 kg is solid food contaminated with melamine [17].

Due to the fact that in foods such as coffee, orange juice, lemon juice and milk, melamine levels up to 2.2 mg/kg were reported, the Food Standards Australia considered the possibility of food contamination with melamine following food contact with plastic packaging and proposed in 2008 a maximum permissible level of 2.5 mg/kg of food [18].

A melamine level higher than this is an indicator of intentionally added melamine in the food product (a product falsification with melamine).

In the USA, the level of security for milk and milk products was set at 2.5 mg/kg, while in Europe, the Food Safety Authority has set the limit at 2.5 mg/kg (ppm, parts per million) for all products containing more than 15% milk [17]. However, for a greater security, a basic level was set well below 2.5 mg/kg at 0.002-13 μg/kg (ppb, parts per billion) [2].

Clinical aspects

Clinical studies on children from China exposed to melamine contamination by milk powder consumption showed the presence of small kidney stones with a high content of uric acid and melamine, without large changes in values of other biochemical analytes (increased blood creatinine, leukocyte increased). The mechanism of action of melamine consisted of obstructive uropathy and acute renal failure in the most severe cases [19]. There was a positive correlation between the size of kidney stones and melamine content of ingested material but not with the duration of exposure [20]. Clinical manifestations which occurred were crying, vomiting, fever, hematuria, disuria, oliguria, anuria, hypertension, edema and pain in the kidney area. Not all children with kidney stones experienced these clinical manifestations. Progressive jaundice liver injury occurred only in a small number of investigated children exposed to melamine contamination [21-30]. It seems that liver damage is due, in part, to blocked bile ducts. Also, lower urinary pH and increased plasmatic levels of free fatty acids can accelerate kidney stones formation.

Melamine is a compound that normally should not be in food and the intentional addition to foods is prohibited, being a toxic compound if inhaled, swallowed or absorbed in the skin and causes eye, skin and respiratory system irritation [16]. Chronic exposure to melamine can cause cancer or reproductive disorders [16].

2. Materials and methods

The aim of this study was to analyze the contamination with melamine of food products. The materials used in the study consisted of chicken meat and powder milk.

We selected six different chicken meat products commercially available which contained chicken legs, with origin in four geographic regions of Romania (Transilvania, Banat, Muntenia and Moldova), in different types of packaging.

Four products contained refrigerated chicken meat packed in sealed plastic trays and the other two contained refrigerated chicken meat packed in sealed plastic bags. The analysis of all products was performed before the expiration date written on the package.

For analysis of melamine contamination in powder milk, four different types of commercially available powder milk formulas were selected, both infant formulas and follow-on formulas.

The meat and powder milk products were prepared for analysis as follows:

- Ten samples of fresh chicken meat were collected from ten different regions of each chicken meat product. The 10 samples were grounded and mixed with a blender machine and 10 g of mixture for each of the 6 chicken products were used for the next step of analysis.
- The powder milk samples were reconstituted with a solution of 10% methanol/PBS according to manufacturer's instructions for different age intervals (Table 1) and 7 samples were prepared.

All chicken meat and milk samples were analyzed according to the manufacturers' protocol with Melamine Abraxis ELISA kit (Biosense Laboratories AB, Norway) on a Stat Fax 2100 (Awareness Technology) ELISA line. The kit is recommended for the detection of melamine in milk powder, whole milk, yogurt, ice cream and chicken meat. The method ELISA is an immunometric technique which can be used for detection of very small quantities of analytes in different types of samples, thus of melamine in food samples.

3. Results and Discussion

The six chicken meat samples were analyzed in triplicate and the results are shown in Table 2. The values we obtained were lower than FDA food limit of 2.5 mg/kg but greater than the security limit of 13 μg/kg specific to environment contamination.

Nevertheless, what should be emphasized is the fact that all the melamine content values are lower than 2.5 ppm which confirms that melamine presence in the chicken meat products is not the result of intentional addition, but may be the result of contamination from food packaging or the environment.

All melamine content values determined for all powder milk samples were lower than the limit set by FDA which is less than 2.5 mg/kg (Table 3).

However, for a greater security, the level of melamine contamination due to general environment should be taken into account (0.002 - 13 μg/kg or ppb) which is a limit set much lower than 2.5 mg/kg [2]. All samples of powder milk formulas had values above these security limits. This indicates the presence of melamine contamination, but the contamination was not the result of an intentional addition because the values were lower than 2.5 mg/kg.

Table 1. Powder milk samples preparation

No. of initial powder milk products	No. of reconstituted samples	Age of the infant	Powder milk samples preparation		
			Volume of 10% MeOH / PBS solution (mL)	Powder milk quantity (g)	Final volume (mL)
Pr. 1 (infant formula)	S1a	< 6 months	30	4.5	31
	S1b	> 6 months	30	4.7	32
Pr. 2 (infant formula)	S2a	< 6 months	30	4.5	31
	S2b	> 6 months	30	4.9	33
	S2c	> 9 months	30	5	35
Pr. 3 (follow-on formula)	S3	> 18 months	30	4.3	30
Pr. 4 (follow-on formula)	S4	> 18 months	30	4.7	32

Table 2. Average values of melamine concentration (media ± standard deviation) for 3 sets (triplicate) of chicken meat samples

Meat sample number	Set I Concentration (ppb)	Set II Concentration (ppb)	Set III Concentration (ppb)	Average concentration of sample (ppb)
CPR1	13.3	12.3	14.4	**13.3±1.1**
CPR2.	16.3	16.1	17.7	**16.7±0.85**
CPR3.	18.4	17.7	19.8	**18.6±1.1**
CPR4.	17.5	17.2	18.8	**17.8±0.9**
CPR5.	20.9	20.5	22.5	**21.3±1.1**
CPR6.	18.7	18.7	20.2	**19.2±0.8**

Table 3. Melamine concentration values (mg/kg) in milk powder samples

Powder milk sample number	Values obtained by ELISA determination (ng/mL)	Sample volume (mL)	Mass of powder milk from the sample(g)	Melamine concentration (ng/g)	Melamine concentration (mg/Kg)
S1a	22.3	31	4.5	153.6	0.15
S1b	32.2	32	4.7	218.9	0.22
S2a	34.8	31	4.5	240.1	0.24
S2b	22.3	33	4.9	149.9	0.15
S2c	21.7	35	5	151.7	0.15
S3	19.5	30	4.3	135.7	0.14
S4	16.3	32	4.7	110.7	0.11

When comparing the concentration of melamine in infant formulas and follow-on formulas, higher melamine concentrations were found in infant formulas, although the mass of solid powder milk in these types of formulas is lesser than in follow-on formulas.

If we are also taking into account the fact that the number of daily meals is greater for the newborns, it is possible that the daily dose of melamine to which newborns are exposed is higher than the dose for children over 6 months.

We calculated the melamine dose to which infants less than 6 months were exposed by consuming powder milk from food producers 1 and 2 (S1a, S1b, S2a, S2b, S2c) which had the highest content of melamine. Thus, considering 600 ml the daily milk volume, prepared using the powder milk formula from company 1, the calculated dose is 0, 0138 mg/day. If we use the powder milk formula of the second company, 2, the calculated dose is 0, 0216 mg/day. These values change if they correlated with the child's weight, but still they are below melamine base concentration (estimated from several sources of contamination) in milk powder formulas [2] to be between 0.54 - 1.6 mg/kg/day. Of the four companies analyzed, 3 are from abroad and one from Romania [2].

4. Conclusions

The aim of this study was the analysis of melamine contamination in food products (chicken meat and milk powder) from different regions of Romania, packed in different types of packaging and produced by different food producers in order to determine melamine concentration in analyzed samples and to observe the influence of food products origin (the region of origin) and type of packaging on the content in melamine. From literature data we have studied, it seems that this is among the first studies of its kind in Romania.

The results we obtained demonstrated the following:

- The screening analysis performed on 6 chicken meat samples and 7 powder milk samples showed the presence of melamine in all the analyzed samples
- The melamine levels in all analyzed samples (13.3 – 21.3 ppb for chicken meat and 110.71 – 240.05 ppb for powder milk) were lower than 840 ppb and lower than the maximum permissible limit of 2.5 ppm (2500 ppb) for food, which shows that the presence of melamine is not the result of intentional addition in these products
- The determined level of melamine in analyzed samples was higher than the level for a greater security, which is a basic concentration level set well below 2.5 mg/kg, respectively at 0.002-13 µg/kg (ppb, parts per billion)
- Larger studies are required to establish correlations between the geographical region, type of packaging and the shelf life period.

Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

References

1. Regulamentul (CE) 178/2002, JO L31/1 din 28.01.2002, http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002R0178:20080325:RO:PDF
2. FAO/WHO Food Standard Programme, Codex Committee on contaminants in food, Proposed Draft Maximum Levels for Melamine in Food and Feed (N13-2009), 4th sesion, Izmir, Turkey, 26-30 April, 2010, http://www.ijhar-s.gov.pl/pliki/iso/SESJE_KKZ/sesje2010/CX.CF% 2010.4.1.pdf
3. WHO, 2008 a, Report from Expert Meeting to Review Toxicological Aspects of Melamine and Cyanuric Acid, Held in Collaboration with FAO and Supported by Health Canada, Ottawa, Canada, 1-4 December, 2008, http://www.who.int/foodsafety/publications/chem/Melamine_ report09.pdf
4. Regulamentul CE 1881/2006, JO L364, 20.12.2006, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri= DD:13:58:32006R1881:RO:PDF
5. Anexa I a Regulamentului CE 1881/2006, JO L364, 20.12.2006, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=DD:13:58:32006R1881:RO:PDF
6. Regulamentul CE 1882/2006 al Comisiei din 19.dec.2006, JO 15/vol18, L364/25, 20.12.2006, http://www.justice.gov.md/file/Centrul%20de%20armonizare%20a%20legislatiei/Baza%20de%20date/Materiale%202012/Legislatie/32006R1882.PDF
7. Regulamentul CE 1883/2006 al Comisiei din 19 dec.2006, JO 15/vol18, L364/32, 20.12.2006, http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32006R1883:RO:NOT

8. Regulamentul CE 401/2006 al Comisiei din 23.februarie.2006, JO 03/vol70, L70/12, 9.03.2006, http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R0401:20100313:RO:PDF
9. Regulamentul CE 333/2007 al Comisiei din 28.martie.2007, JO L88/29 din 29.03.2007, http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:088:0029:0038:RO:PDF
10. Ishiwata H., Inoue T., Tanimura A., Migration of melamine and formaldehyde from tableware made of melamine resin, *Food Additives and Contaminants*, 1986, *3*(1), 63-70, PMID: 3956795
11. Bradley EL et al., Survey of migration of melamine and formaldehyde from melamine food contact articles available on the UK market, *Food Additives and Contaminants*, **2005**, *22*(6), 597-606, PMID: 16019835
12. Lund KH, Petersen JH, Migration of formaldehyde and melamine monomers from kitchen and tableware made of melamine plastic, *Food Additives and Contaminants*, **2005**, *23*(9), 948-955, doi: 10.1080/02652030500415660
13. WHO, 2008 b, Background Paper on Occurence of Melamine in Food and Feed prepared for the WHO Expert Meeting to Review Toxicological Aspects of Melamine and Cyanuric Acid, Held in Collaboration with FAO and Supported by Health Canada, Ottawa, Canada, 1-4 December, 2008
14. Patakioutas D et al., Application and fate of cycromazine in a closed cycle hydroponic cultivation of bean (*Phaseoulus Vulgaris* L.), *Journal of Agricultural and Food Chemistry*, **2007**, *55*(24), 9928-9935, doi: 10.1021/jf071726i
15. Karras G. Et al., Fate of cycromazine applied in nutrient solution to a gerbera (*Gerbera jamesonii*) crop grown in a closed hydroponic system, *Crop Protection*, **2007**, *26*(5), 721-728, http://dx.doi.org/10.1016/j.cropro.2006.06.011
16. Melamine in milk by David Bradley, Sciencebase, 17sept 2008, http://www.sciencebase.com/ science-blog/melamine-in-milk.html
17. D.P.H.Hsieh et al., Toxicological analysis points to a lowertolerable daily intake of melamine in food, *Regulatory Toxicology and Pharmacology*, **2009**, *55*(1), 13-16, doi: 10.1016/j.yrtph.2009.05.004
18. Food Standards – Australia New Zeeland, Risk assesement and referral levels for dairy foods and foods containing dairy-based ingredients adulterated with melamine, 14 Oct. 2008, http://www.foodstandards.gov.au/scienceandeducation/factsheets/factsheets2008/melamineinfoodsfromchina/riskassessmentandref4064.cfm
19. WW TU et al., One-year Follow Up of Melamine-associated Renal Stones in Sichuan and Hong Kong, *HK J Paediatr* (new series), **2012**, *17*, 12-23
20. Hu P, Lu L, Hu B, Zhang CR, The size of melamine-induced stonesis dependent on the melamine content of the formula fed, but not onduration of exposure, *Pediatr Nephrol*., **2010**, *25*(3), 565 – 566, doi: 10.1007/s00467-009-1351-2
21. Hu P., Wang J. Et al., Liver Involvement in Melamine-associated Nephrolithiasis, *Arch.Iran Med.*, **2012**, *15*(4), 247-248
22. Liu et al., Recent developments in the detection of melamine, *J. Zhejiang Univ.-Sci.B(Biomed&Biotechnol.)*, **2012**, *13*(7), 525-532, doi: 10.1631/jzus.B1100389
23. WHO, 2009b, Background paper on methods for the analysis of melamine and related compounds in foods and animal feeds, http://www.who.int/ foodsafety/fs_management/ Melamine_3.pdf
24. Squadrone, S., Ferro G.L., Mauro DMC, Determination of melamine in feed: validation of a gass chromatography-mass spectrometry method according to 2004/882/CE regulation, *Food Control*, *21*, 714-718, doi:10.1016/j.foodcont.2009.10.013
25. Miao H. et al. Simultaneous determination of melamine, ammelide, ammeline, and cyanuric acid in milk and milk products by gass chromatography tandem mass spectrometry, *Biomed. Environ.Sci*., **2009**, *22*(2), 87-94, http://dx.doi.org/10.1016/S0895-3988(09)60027-1
26. Ibanez M. Et al., Determination of melamine in milk- based products and other foods and beverage products by ion-pair liquid-chromatography-tandem mass spectrometry, *Anal. Chim. Acta*, **2009**, *649*(1), 91-97, http://dx.doi.org/10.1016/j.aca.2009.07.016
27. Venkatashami, G., Sowa RJJ, A rapid acetonitrile-free HPLC method for determination of melamine in infant formula, *Anal. Chim. Acta*, **2010**, *665*(2), 227-230, http://dx.doi.org/10.1016/j.aca.2010.03.037
28. Yang HH et al., Moleculary imprinted polymer as SPE sorbent for selective extraction of melamine in dairy products, *Talanta*, **2009a**, *80*(2), 821-825, http://dx.doi.org/10.1016/j.talanta.2009.07.067
29. Guo et al., Visual detection of melamine in milk products by label-free gold nanoparticles, *Talanta*, **2010**, *82*(15), 1654-1658, http://dx.doi.org/10.1016/j.talanta.2010.07.035
30. Garber E.A., Detection of melamine using commercial enzyme-linked immunoabsorbent assay technology, *J.Food Prot*, **2008**, *71*(3), 590-594